ON THE PRODUCTION OF MONSTERS BY HYBRIDIZATION.

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Of late there have appeared several papers that deal with the production of monsters in teleosts, especially in the common mud-minnow, *Fundulus heteroclitus*. The papers of Kellicott ('16) and Werber ('15 and '16) have been especially interesting because they deal with the same type of monster that I have been studying incidentally for more than ten years and that I have described and discussed in several different papers dealing with development and heredity in fish hybrids.

In 1908 I published the results of intercrossing *Fundulus heteroclitus* and *F. majalis* (Newman, '08). In the cross *F. heteroclitus* × *F. majalis* I called attention to the various types of monsters, noting especially those types in which the head and eyes developed and the body was relatively inhibited, and those in which head development was inhibited and body was relatively normal.

The wide range of variability in the results of crossing two individuals of different species was emphasized. A long list of conditions was given ranging from extreme subnormal eggs that failed to cleave, up to markedly supernormal individuals that are hardier and better equipped to live than individuals of either pure strain. In subsequent papers (Newman, '12 and '14) I called further attention to the graded series of teratomata resulting from one set of eggs fertilized by sperm from a single male.

In general it was shown that in hybrids between closely allied species (homogenic hybrids) the rate of development of a considerable percentage of individuals was accelerated, but that in all heterogenic hybrids there is a more or less marked retardation of developmental rate from the earliest cleavage stages on. Even in the case of homogenic hybrids, the retarded individuals are always abnormal.

During the summers of 1914 and 1916 a series of experiments was performed in order to test the theory that retardation is always correlated with abnormal development. In hybrid experiments in which *Fundulus heteroclitus* eggs are fertilized by the sperm of the mackerel there is noticeable in comparatively early cleavage stages a marked difference in number and uniformity of blastomeres in different eggs of the same batch. The different types of eggs were divided into three lots as follows:

A. Those that were most advanced and exhibited the largest number and greatest regularity of cells.

B. Those in which the cells were fewer and less uniform.

C. Those in which the cells were relatively fewest and of various sizes.

The development of these three lots was watched from day to day with the result that all of those in A reached a fairly advanced condition before abnormalities appeared and abnormalities concerned chiefly the eyes and heart; those in B showed all sorts of monsters including cyclopeans, eyeless types, and those with reduced bodies; those in C produced solely apical parts without bodies, amorphous masses of tissue or at best isolated organs such as eyes and hearts.

The converse of this experiment was tried using the eggs of *F. heteroclitus* and the sperm of the closely related *F. diaphanus*. In this experiment there was very little acceleration noticeable during the cleavage stages, but during gastrulation it was a simple matter to separate out three classes A, B, and C as follows:

A. Those in which the germ ring was distinctly more than half way round the yolk.

B. Those in which the germ ring was approximately half way round the yolk. These were about like the control.

C. Those in which the germ ring was distinctly less than half way round the yolk.

The eggs in lot A nearly all hatched on the average distinctly earlier than the control (pure *F. heteroclitus*), and were distinctly more active, grew faster and lived longer than the control. Those in lot B showed a wide range of variability, some hatching as early as the control, others hatching later, and still others failing to hatch. Various common types of abnormality oc-

curred but none of the most extreme types were noted. Those in lot C showed the most pronounced abnormalities. Only a few hatched and these were rather feeble. The remainder were monsters belonging to a wide range of types, many showing merely some defect in the circulation (string-hearts, enlarged pericardia, no blood, etc.), others being defective in the head parts, especially eyes, and others being defective chiefly in the posterior parts.

These two experiments were repeated many times and gave substantially identical results each time. There can be no question therefore that *there is a very close correlation between the rate of development and the degree of normality of the embryos.*

When in homogenic crosses a slight increase in rate of development is instituted the result is a supernormal F_1 hybrid type, in which the difference is largely physiological, consisting of greater activity and greater viability. When in the heterogenic crosses there results a more or less pronounced retardation in developmental rate (sometimes clearly seen in the early cleavage stages) a subnormal type or monster is the result, which differs from the normal not only physiologically in being non-viable but morphologically in being defective in one or more parts.

Both supernormal and subnormal types appear then to be correlated with an alteration in the normal rate of development.

It is quite evident from numerous experiments (Newman, '15) that the degree of retardation resulting from varous heterogenic hybridizations is not a factor of the distance of the cross, for some species of the same genus cross with poor success, and some species of different orders of teleosts cross so as to produce swimming larvae. This argues against the theory that foreign sperm introduces toxins, since the protoplasm of distantly related forms ought to be more toxic than that of near relatives.

About all we can say then is that *in introducing a foreign sperm into an egg we either accelerate or retard the developmental rate of the egg.* In proportion as the rate is accelerated we get a supernormal result, and in proportion as the rate is retarded, a subnormal result.

That there is nothing specific about the effect of foreign sperm upon the developmental rate of the egg is further shown by the

fact that the same series of subnormal types (monsters) are found in a great variety of different crosses, and also by the fact that these same abnormal types may be produced by purely physical or by chemical agents.

Other Methods of Producing Monsters.

As long ago as 1907, Stockard, by the use of solutions of magnesium chlorid, was able to produce, among other abnormal conditions, a considerable percentage of cyclopic and allied ophthalmic terata. Although little was said about abnormal conditions other than those associated with the particular terata mentioned, enough data was given to show that many if not all of the other well-known conditions occurred.

In 1909 and 1910 Stockard contributed further papers concerning the development of teratomata, showing that the same results could be obtained with alcohol and other anæsthetics. He called particular attention to ophthalmic terata, making it clear that the eye anlages appear to be especially susceptible to anæsthetics, and explained cyclopia and similar conditions as "the result of an anæsthetic action during the early developmental stages." Later, when McClendon ('12) obtained a similar series of eye defects by the use of substances that are non-anæsthetic in action, Stockard took the more general view that deleterious chemical substances merely lower the vitality of certain sensitive anlages and cause arrested development of important structures. Stockard implies that the results are primarily due to a lowering of developmental rate. The paired eyes for example are believed to arise from an unpaired median anlage which normally undergoes a sort of twinning process producing two eyes. Under the influence of deleterious chemicals "the median anlage does not widen or spread laterally but is arrested in its primary median condition." Hence the unpaired cyclopean eye develops.

Recently Werber (1915, 1916) in three papers and especially in his latest one (1916) re-attacked the problem from a somewhat novel point of view. Starting in with the assumption that the development of monsters in mammals is caused by "pathologic parental metabolism" he decided to test his hypo-

theses with fish embryos. He consequently subjected the embryo of the fish *Fundulus* to solutions of various metabolic by-products, characteristic of normal and pathologic mammals, such as urea, butyric acid, lactic acid, acetone, sodium glycocholate, and ammonium hydroxide. Conclusive results were obtained only with butyric acid and acetone, especially the latter. The paper is especially valuable for its comprehensive record of terata, their classification, and the numerous figures of monsters of every sort. All of the results are similar to those obtained in hybridization experiments, as is evidenced by the fact that the illustrations would serve as well for a paper on hybrid teratomata as for the paper in which they appear. All of Stockard's teratomata are also duplicated exactly.

It is especially to be noted that there are two markedly different types of monsters: (*a*) those in which the head parts alone are inhibited, and especially the eyes and heart, and (*b*) those in which the head parts develop fairly normally and the posterior parts are inhibited. Extremes of (*a*) are seen when only the eyes or heart are effected, and of (*b*) when eyes or heart develop alone to the exclusion of other parts of the body. These results appear at first sight quite paradoxical, but the explanation will be clear from what follows.

Werber appears to realize the importance of Child's theory of the "axial gradient" in organisms as an aid in understanding the morphogenesis of monsters. He points out that the anterior end, or "apical end" is most susceptible to agents that inhibit development, and hence we have a logical basis for the frequency of ophthalmic terata. He sees in Child's work however no explanation of the occurrence of heads with reduced body and of isolated eyes, hearts, etc.

Werber's idea of the morphogenesis of monsters differs somewhat from that of Stockard in being strongly morphological. According to him any chemical substance, whether anæsthetic, toxic, or what not, so long as it is injurious to life, checks the process of cell metabolism and cell division and causes the affected cells to *disintegrate.* This "*differential blastolysis*" accounts to Werber's mind for the suppression of certain anlages, especially of those at the apical end of the embryo; but it fails to account

for the development of isolated eyes and hearts in other embryos of the same series, which are treated exactly as those were in which the eyes were suppressed or the heart failed to develop.

It will be seen that both Stockard and Werber believe that abnormal development is the result of toxic or deleterious substances introduced into the egg from without. It has been shown however that identical results can be obtained by purely physical methods.

In a very recent paper Kellicott ('16) has reported the *production* of every known type of teratomata in *Fundulus* by merely subjecting very young embryos to low temperatures. Eggs placed in the refrigerator a few minutes after insemination very nearly stopped development and some apparently underwent regressive changes. When returned to normal temperatures these eggs developed nearly every type of abnormality. The most frequent terata were those of the eyes and of the heart. A list of terata would be a mere repetition of those obtained by other methods.

By way of a theory of the genesis of monsters Kellicott proposes what he calls a "disorganizational hypothesis," namely "that the cause of abnormal and monstrous development, is to be found in a disturbance of the normal organization of the ovum, as expressed by the unusual character and distributions of the differentiated materials of the egg protoplasm." It should be noted in connection with these low temperature experiments that the first result is a very pronounced lowering of developmental rate, amounting in some cases almost to a cessation of development, accompanied by certain regressive changes. There is also in all cases a permanent retardation even after the embryos are returned to normal temperatures. It seems then that *the primary effect of low temperature is the lowering of metabolic rate.*

It will now readily be seen that the whole series of teratomata in fish eggs may be produced in three entirely different ways, the results being the same in each:

1. By the use of chemicals whose action is such as to lower the rate of metabolism.

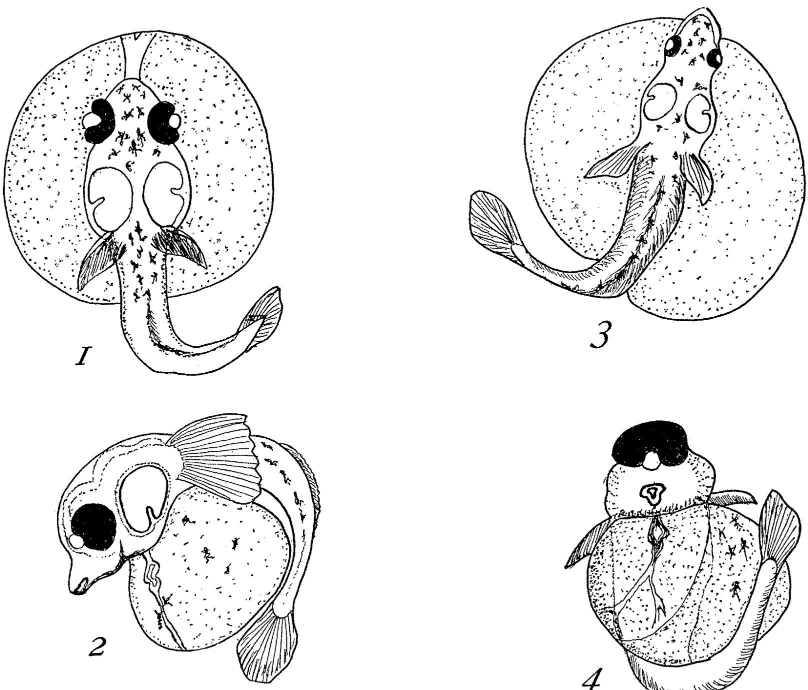
2. By means of low temperatures, which effects directly the rate of metabolism.

3. By means of heterogenic hybridization, which always effects a lowering in the rate of development.

All monsters then seem to be the result of a lowering of developmental rate.

Let us now return to a consideration of hybrid monsters and ask ourselves the question: *how does the foreign sperm lower the rate of development?* Various views are held as to the way in which the foreign sperm retards development.

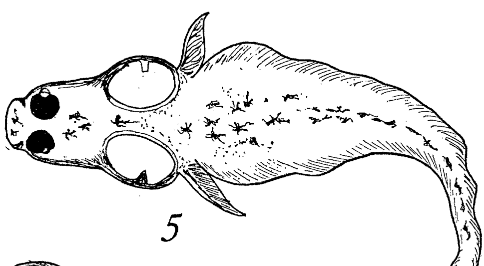
Moenkhaus holds that the foreign sperm exercises a poisonous

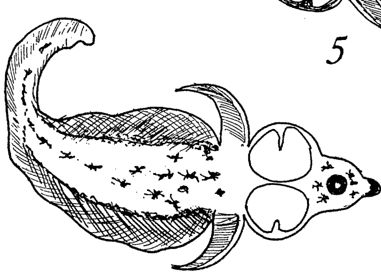


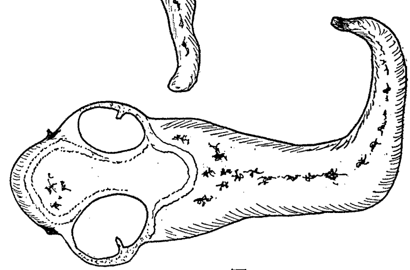
or toxic effect in proportion to the distance of the cross. Loeb holds that the results are due to the inability of the hybrid to digest the yolk with sufficient rapidity. I have expressed the view that there is an incompatibility between the nuclear elements of the two different species that results in abnormal chromosome arrangements and irregular distributions of the latter to various cells. Undoubtedly also the cleavage mechanism works less perfectly when chromosomes of very different size

and form are mixed together in a single cell, than when they are all of the same kind. Whatever the primary cause of abnormal development may be, the most obvious early effect is a lowering of developmental rate and it is on this point that the similarity exists among all of the experimental results heretofore discussed.

Since we are dealing primarily with rates of metabolism, we shall doubtless find, as Werber has suggested, that the morphogenesis of monsters in teleosts is simply a special case of form regulation similar to those recently described and discussed by Child ('15 and '16) for sea-urchins, in which he demonstrates the effectiveness of the axial metabolic gradients as dynamic factors in the development of various types of monsters. He has been able to "control and modify development by means







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of the differential action of external factors on different regions of these gradients."

It is my belief that an application of the principles enunciated by Child serve to rationalize the results of heterogenic hybridization as well as those produced by chemicals and by low temperatures, and since Werber merely suggested the possibility of explaining ophthalmic anomalies by this theory and failed to apply it in any far-reaching way to the other various types of monster he was dealing with, it seems worth while to give the theory a thorough trial in this field.

The Axial Gradient Theory and the Morphogenesis of Monsters Resulting from Heterogenic Hybridization.

The axial gradient in vertebrates is essentially a quantitative one depending on the rate of metabolic activity. The rate along the apico-basal axis is highest at the anterior or apical end and lowest at the posterior or basal end. Paralleling the axial metabolic gradient there exists a susceptibility gradient, in which the apical end is most susceptible to agents that lower the vitality or retard the rate of metabolism. Similarly the median dorsal region is the most susceptible point of the bilateral or mesiolateral axis and the lateral part the least susceptible. Certain points that are both apical and median will be the most susceptible of the whole system. The eyes are such structures developed from a primitive mesio-dorsal-apical anlage, and are therefore the most susceptible of all parts of the body.

In animals of the high grade of complexity exhibited by vertebrates there are to be distinguished several semi-independent axiate systems. Undoubtedly the central nervous system and its derivatives constitutes an axiate system by itself. Similarly the circulatory system is axiate with the heart the apical point and the veins and arteries basal. The skeleton with the fin systems are also semi-independent axiate systems, with the anterior parts more susceptible than the basal ones. This is brought out in recovery cases in which the pectoral fins recover while the pelvic do not.

Whenever young fish embryos are subjected to any of the types of inhibiting or retarding agents that have been previously discussed we are able to classify the resulting abnormal forms into categories (modifying somewhat the usage of Child): (*a*) Forms in which there has occurred an inhibition of anterior and of median dorsal regions; (*b*) Forms in which these regions have become acclimated or have recovered from an initial inhibition. Curiously enough in chemical experiments those parts that are most susceptible to inhibiting agents of higher concentrations are able most completely to acclimate themselves in lower concentrations or to recover when the inhibiting agent is removed or its severity lessened. Let us consider these two categories of teratomata in some detail.

(A) Forms Resulting from Differential Inhibition.

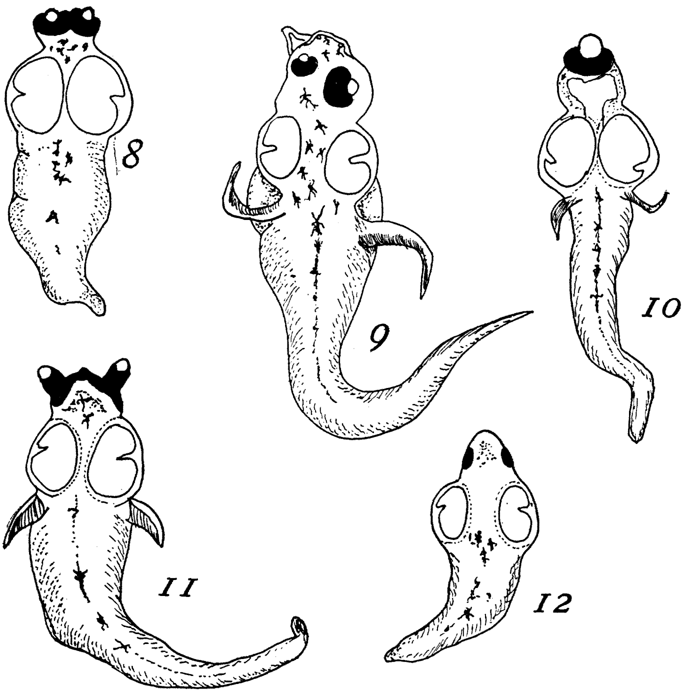
In fish monsters, whether they are the result of chemical, physical, or biological (hybridization) inhibitors, the most prevalent abnormalities are associated with the eyes and with the heart. The teratomata resulting from all three types of inhibiting agents are strikingly similar, so I shall from now on confine my description and discussion to monsters produced by heterogenic hybridization. In any heterogenic cross the least abnormal forms are always those that have something slightly abnormal or sub-normal about the eyes or the heart. We must conclude then that the primordia of these two structures are peculiarly susceptible to agents that retard metabolic rate.

Ophthalmic Terata.—Eyes in the least extreme cases may be either a little too small, a little too close together, of unequal size, or asymmetrical in position. In the most extreme cases the eyes may be wanting, or very poorly differentiated; the eyes may be fused more or less completely (various grades of cyclopia) or there may be a single unilateral eye. Every intergrade between the least extreme and the most extreme ophthalmic terata may be readily found in almost any hybrid experiment. Why in a single batch of eggs there should be so highly diversified a result is not known, but it must be due to physiological (age, maturity, etc.) differences in the individual eggs or sperm, or a combination of both.

In general we may conclude that suppressed eyes, either bilateral or unilateral, are the result of inhibition primarily of the apical end of the apico-basal gradient, while eyes too close together or fused in the median line (cyclopic terata) are the result primarily of inhibition of the median portion of the mesio-lateral axis.

Cardiac Terata.—All grades of heart abnormalities occur in heterogenic crosses. The less extreme cases are those in which the heart development is belated relatively to the other bodily regions, or in which there may be a failure of the whole cardiac mechanism to become inclosed within the body cavity. In such cases the heart remains outside the body and there is usually a much enlarged pericardium. In the most extreme cases the heart never develops, or becomes a mere pulsating strip of muscu-

lar tissue running from the body to the yolk of the egg across the pericardium. These "string-hearts" are among the commonest terata to be found in heterogenic hybrids. We have no choice but to conclude that, although the heart is not very close to the apical end, and is ventral rather than dorsal in position, that it



is an organ of relatively high rate of metabolism and shows extreme susceptibility to retarding agents. It is undoubtedly true that the heart and blood vessels form an axiate system of their own, which is somewhat independent of general somatic axes. The attached or arterial end (ventricles) of the heart is the apical point of this axis and is the part most susceptible to retarding agents. When this end of the heart is inhibited the whole circulatory system will be rendered functionless, though the basal parts may be fairly well developed. When the apical parts are only slightly abnormal the basal parts may be fully normal, but when the apical parts show extreme abnormality the basal parts are usually abnormal but less so than the apical.

(B) Forms Resulting from Differential Acclimation or Recovery.

Of somewhat less frequent occurrence than the above described types of fish hybrid teratomata are those in which the apical and mesial parts have become acclimated to (or adjusted to) the materials brought in by the foreign sperm. The less extreme types are those in which the head is relatively large and wide as compared with the trunk and tail. Next comes a whole series of types in which first the tail and then the trunk are inhibited more or less completely, and even heads without trunks but with beating hearts are common. The most extreme cases are those in which isolated eyes or isolated hearts grow upon an otherwise undifferentiated blastoderm (Fig. 14). I have found several examples of isolated eyes that could be distinguished only by the presence of a lens and of pigmented retinal cells. Also isolated hearts may be reduced to small pulsating "drums" of tissue in which there is no axiate elongation. According to the axial gradient theory not only are the apical points most susceptible to inhibitors, but under certain circumstances, such as long exposure to low concentrations, they have the highest capacity for recovery. That is why eyes and hearts, the apical parts of the two axiate systems, show the highest capacity for recovery and are sometimes the only parts that do recover after long and general inhibition. A very common type of teratomata is one in which the whole body is decidedly abnormal, inhibited no doubt at an early stage, but in which recovery of the apical structures has occurred to various degrees, so that we have forms in which the only highly differentiated parts are apical parts. Such embryos are shown in Figs. 13 and 14.

Child ('16) distinguishes between forms resulting from differential acclimation, forms resulting from differential recovery, and forms resulting from differential inhibition with general recovery. While I believe that the forms resulting from hybridization are identical to those produced by chemical inhibition, I am not able to say in any particular case whether a form is one or the other of these three types. My judgment is that the isolated eyes and hearts are forms resulting from differential recovery and that the forms with comparatively well developed

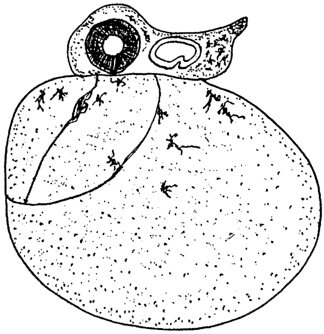
head and small or rudimentary body are the result of differential acclimation. After all, the results of acclimation and recovery even in controlled chemical experiments are so nearly identical that, were it not for the differences in experimental method used, they would be indistinguishable.

On the whole then we can distinguish clearly only the two types of monsters:

(*a*) Forms with inhibited apical parts and relatively well developed basal parts—the results of differential inhibition.

(*b*) Forms with inhibited basal parts and relatively well developed apical parts—the results of differential acclimation or recovery.

None of the forms of fish hybrids with which I am acquainted fail to fall into one of these two categories and to receive a logical



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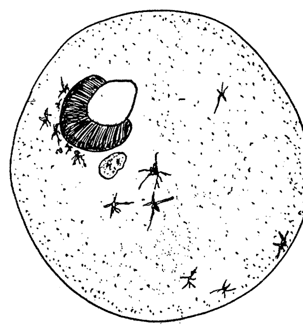

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explanation on the basis of the axial gradient theory. It is my belief that this theory offers the best explanation of teratological development in fish and in all other axiate forms, including man.

As a concrete illustration of the application of the theory to a single experiment, I shall offer the details of an intensively studied hybridization experiment, the one referred to earlier in the paper in which the three lots of hybrid eggs were separated on the basis of the rate of early cleavage into three lots A, B and C. This experiment dealt with hybrids of *Fundulus heteroclitus* ♀ × *Scomber scombrus* (mackerel) ♂.

Figs. 1–4 show types of teratomata in lot A. These embryos are frequently almost normal up to an advanced stage and a

very few hatch as perfectly normal larvæ. A considerable percentage of embryos develop a circulation but have comparatively small eyes as in Fig. 1. Others have a proboscis and the eyes both look forward as in Fig. 2. Still others have a very narrow head with small eyes and the heart is a string-heart as in Fig. 3. Others, finally, are cyclopic forms in which the mesio-lateral development of the head has been more seriously inhibited than the apico-basal (as in Fig. 4).

The largest and most varied assemblage of monsters is found in Class B in which the rate of cleavage was intermediate between the lowest and highest rate. Types of embryos of this group are shown in Figs. 5–12, which are camera lucida drawings made when embryos were two weeks old. In all of these the whole body from head to tail is abnormal (compare with the embryos of A in which the tail is nearly normal). Ophthalmic terata are most common here. Fig. 5 is microphthalmic and a little asymmetrical and the body and tail are fairly normal; pectoral fins are also developed and the otic vesicles are only slightly enlarged. Fig. 9 has decidedly asymmetrical eyes, one very small; the jaws, pectoral fins, etc., are also asymmetrical, but the tail is fairly well developed. Fig. 11 shows a peculiar type of stalked-eye, which is rather common; body is only slightly abnormal; pectoral fins normal. Figs. 6 and 10 are two types of cyclopic monster with reduced and shortened tail, but with enlarged otic vesicles and good pectoral fins. Fig. 8 is a synophthalmic type with enlarged otic vesicles and much reduced body. Fig. 12 is a common form with much reduced eyes, well developed otic vesicles and small body. Fig. 7 is an eyeless type in which the otic vesicles seem to have usurped the place of the eyes in the body; the body is fairly well developed. All of the types here shown had the heart beating. All of these forms I look upon as products of acclimation after an early partial inhibition. They were not as much retarded as those in lot C. I look upon the enlargement of the otic vesicle as the result of the suppression or partial suppression of the dominant region anterior to it. The otic region now is to some extent physiologically isolated and thus released to grow to a larger size than normal.

I shall show only two types out of a very large number found in lot C in which the slowing of the cleavage rate was most pronounced. Fig. 13 shows a common type which is practically a head without a body. The little up-turned stump is all there is of the body; the heart is still beating; the eyes are in an advanced stage of differentiation. Fig. 14 shows a case of solitary or isolated eye. No other tissues are differentiated. I look upon these as cases of recovery of the apical regions after rather severe early inhibition. There is no hard and fast line to be drawn between the three classes of monsters shown.

It may be said in closing that each type of hybrid cross differs from others in the particular array of monsters displayed. In some there is never cyclopia, but always microphthalmia. In some the extreme recovery types, like that shown in Fig. 13, are much the most common. In many respects the *F. heteroclitus* ♀ × mackerel ♂ cross is the best adapted for the study of the morphogeny of teratomata because of the very wide range of forms produced.

In conclusion I wish to express my appreciation of the far-reaching applications to morphogenetic phenomena of the axial gradient idea as worked out by Child. Few generalizations known in biological science serve to rationalize so wide a range of developmental phenomena.

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